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Effects of an abasic site on triple helix formation characterized by affinity cleaving

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ABSTRACT

The stability of triple helical complexes of pyrimidine oligodeoxyribonucleotides containing one abasic 1,2-dideoxy-D-ribose (ϕ) residue was examined by affinity cleaving. Within a pyrimidine third strand, the triplets ϕ·AT, ϕ·GC, ϕ·TA and ϕ·CG are significantly less stable than the triplets, T·AT, C+GC and G·TA. The decrease in binding produced by an abasic residue is similar to that observed with imperfectly matched natural base triplets, with ϕ·AT and ϕ·GC being less stable than ϕ·TA and ϕ·CG triplets for the sequences studied.

INTRODUCTION

Oligodeoxyribonucleotide-directed triple-helix formation offers a chemical approach for the sequence-specific recognition of double-helical DNA that is more specific than restriction enzymes (1–4). Because triple-helix formation by pyrimidine oligodeoxyribonucleotides is limited to predominantly purine tracts (T·AT, C+GC triplets), it is desirable to find a general solution whereby oligodeoxyribonucleotides (or other analogs) could be used to bind all four base pairs of intact duplex DNA (37°C, pH 7.0). Approaches toward such a goal include the search for other natural triplet specificities (5,6), the synthesis of oligonucleotides capable of binding alternate strands of duplex DNA (7), the design of nonnatural bases for completion of the triplet code, and the incorporation of null residues for skipping (nonreading) base pairs for which matched base triplets are not known.

1,2-Dideoxy-D-ribose (ϕ), possessing a furanose ring with the same stereo-chemistry at C-3′ and C-4′ as that of the natural nucleosides, substitutes a hydrogen atom in place of a base in a triple helical complex. We address here whether this abasic residue in the third strand of a local triple helix could be used as a null position. Recent, calorimetric data suggest that the average enthalpic stabilization for T·AT and C+GC triplets is 2.0 kcal/mol (8). The incorporation of a 1,2-dideoxy-D-ribose (ϕ) residue in the third strand would result in loss of the two stacking interactions that flank the modified position as well as hydrogen bonding between bases at that location in the major groove of DNA. The influence of abasic sites in Watson–Crick double helical DNA has been characterized by spectroscopic and calorimetric techniques (9–12). NMR studies by Patel and coworkers reveal that duplex DNAs containing a single abasic site across an adenine residue are capable of maintaining a right-handed double helical complex with structural perturbations at phosphates near the abasic site (10). A second study reveals a sequence dependent looping out of pyrimidine bases and abasic residues when placed across each other (11). From calorimetric investigations, an abasic site in duplex DNA dramatically reduces the duplex stability, transition enthalpy and entropy (12). Breslauer and coworkers estimate that the magnitude of such lesion induced effects are greater than one would expect based on simple nearest neighbor considerations and that such effects may thermodynamically extend beyond the localized lesion. We report the effect of pyrimidine oligodeoxyribonucleotides containing abasic (ϕ) sites on triple helix stability characterized by affinity cleaving. The relative stabilities of ϕ·AT, ϕ·GC, ϕ·TA and ϕ·CG are compared with those of the other 16 possible base triplets formed by the common bases A, T, G, and C (5).

MATERIALS AND METHODS

Synthesis of oligodeoxyribonucleotides-EDTA

The fully protected oligodeoxyribonucleotides (1 μmol) were synthesized on a Beckman System 1 Plus oligonucleotide synthesizer using standard β-cyanoethyl phosphoramidite chemistry. The 5′-O-DMT-protected thymidine EDTA (T*) triethylester (13) and 1,2-dideoxy-D-ribose (ϕ) nucleoside (14) phosphoramidites were prepared according to published methods. Deprotection of the polymer-bound oligonucleotide and ethylester hydrolysis were accomplished by treatment of the support with 0.1 M NaOH (1.5 mL, 55°C, 24 h). The supernatant was neutralized (~6 μL of acetic acid), desalted (Sephadex G10–120) and lyophilized. The crude oligodeoxyribonucleotides-EDTA were purified by denaturing 20% polyacrylamide gel electrophoresis. Dialysis against water gave pure oligodeoxyribonucleotides-EDTA **1–5** in 20–25% isolated yield.

Cleavage of oligodeoxyribonucleotide 30-mer duplexes

The cleavage reactions were carried out by combining a 2 min preincubated mixture of oligonucleotide-EDTA (1 μM), spermine-4HCl (1 mM), and Fe(II) (25 μM, $Fe(NH_4)_2(SO_4)_2.6H_2O$) with

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the 30-oligomer duplex labeled with ^{32}P at the 5′ end in a solution of tris-acetate, pH 7.0 (25 mM), NaCl (100 mM), calf thymus DNA (100 μM in base pairs) and 40% ethanol. After equilibration at 27°C for 1 h, the cleavage reactions were initiated by addition of dithiothreitol (DTT) (4 mM) giving a total reaction volume of 20 μL. The reactions were allowed to proceed for 8 h (27°C), stopped by freezing and lyophilization and the cleavage products were analyzed by gel electrophoresis. Individual bands were quantitated by densitometry and scintillation counting.

RESULTS AND DISCUSSION

The relative affinity of the abasic nucleoside (φ) for all four base pairs within a pyrimidine triple helix motif was examined by affinity cleaving. Oligodeoxyribonucleotides **1**–**5** equipped with the DNA cleaving moiety, EDTA·Fe(II) (T*) at a single thymidine position and differing at one base position d(T_7NT_7) (N=T, C, A, G, or φ) were prepared in order to compare the relative stabilities of triple helical complexes with 30-bp DNA duplexes containing the 15 base pair target sites d(A_7XA_7)·d(T_7YT_7) (XY = AT, GC, CG, or TA) (Fig. 1). The 30-bp oligodeoxyribonucleotide duplexes were labeled with ^{32}P at the 5′ end of the Watson–Crick strand d(T_7YT_7). Reaction conditions were chosen to distinguish between stabilities of the variable base triplets (pH 7.0, 27°C, 40% ethanol) (1, 5). The most efficient cleavage was observed for oligodeoxyribonucleotide complexes containing the base triplets T·AT, C+GC and G·TA at the variable position (Fig. 1, lanes 3, 8 and 14). Oligodeoxyribonucleotide **5** containing one 1,2-dideoxy-D-ribose residue (φ) produced moderate to weak cleavage, indicating overall weaker binding for φ·AT, φ·GC, φ·TA and φ·CG triplets presumably due to loss of base stacking and hydrogen bonding. These results are similar to those previously observed for the thirteen additional natural triplet mismatches (5).

Within the pyrimidine triple helix motif, there exists a preference for TA and CG over AT and GC base pairs across the abasic site (φ·TA, φ·CG > φ·AT, φ·GC) (Fig. 2). We cannot distinguish at this time whether this is due to local sequence dependent conformational effects near the variable position in the duplexes as well as the triple helical complexes or differences in the thermodynamic baselines for the four binding sites due to changes in nearest neighbor interactions in the Watson–Crick duplex (Fig. 1, lanes 19–22 and Fig. 2). The decrease in

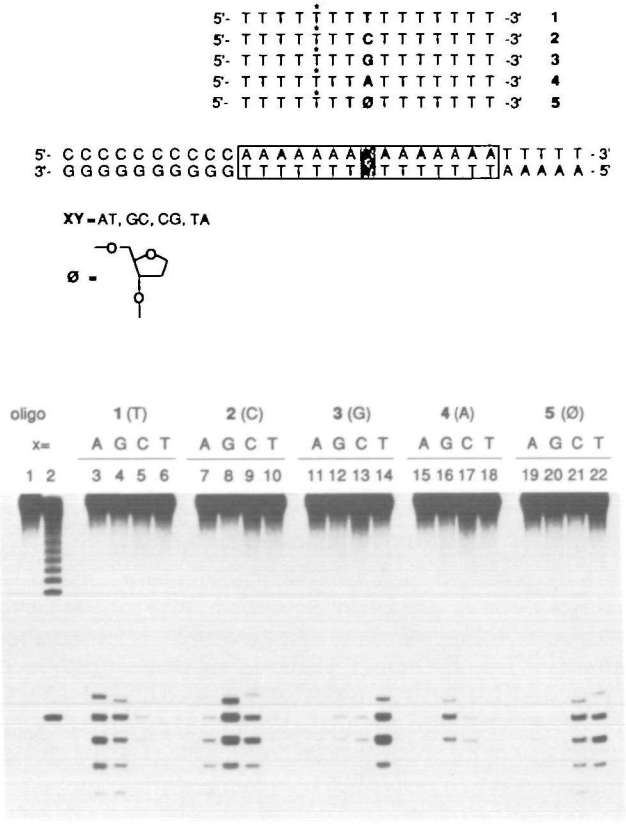

Figure 1. (Top) Sequence of oligonucleotide-EDTA **1**-**5**, where T* is the position of thymidine-EDTA. The oligonucleotides differ at one base position indicated in bold type. The box indicates the double stranded sequence bound by oligonucleotide-EDTA·Fe **1**–**5**. The Watson–Crick base pair (AT, GC, CG, or TA) opposite the variable base in the oligonucleotide is shaded. (Bottom) Autoradiogram of the 20% denaturing polyacrylamide gel. (Lanes 1 to 22) Duplexes containing 5′ end-labeled d($A_5T_7YT_7G_{10}$). (Lane 1) Control showing intact 5′ labeled 30-bp DNA standard obtained after treatment according to the cleavage reactions in the absence of oligonucleotide-EDTA; (lane 2) products of Maxam–Gilbert G+A sequencing reaction; (lanes 3 to 22) DNA cleavage products produced by oligonucleotide-EDTA·Fe(II) **1**–**5**; **1** (lanes 3 to 6), **2** (lanes 7 to 10), **3** (lanes 11 to 14), **4** (lanes 15 to 18), **5** (lanes 19 to 22). XY=AT (lanes 3, 7, 11, 15 and 19); XY=GC (lanes 4, 8, 12, 16 and 20); XY=CG (lanes 5, 9, 13, 17 and 21); XY=TA (lanes 6, 10, 14, 18 and 22).

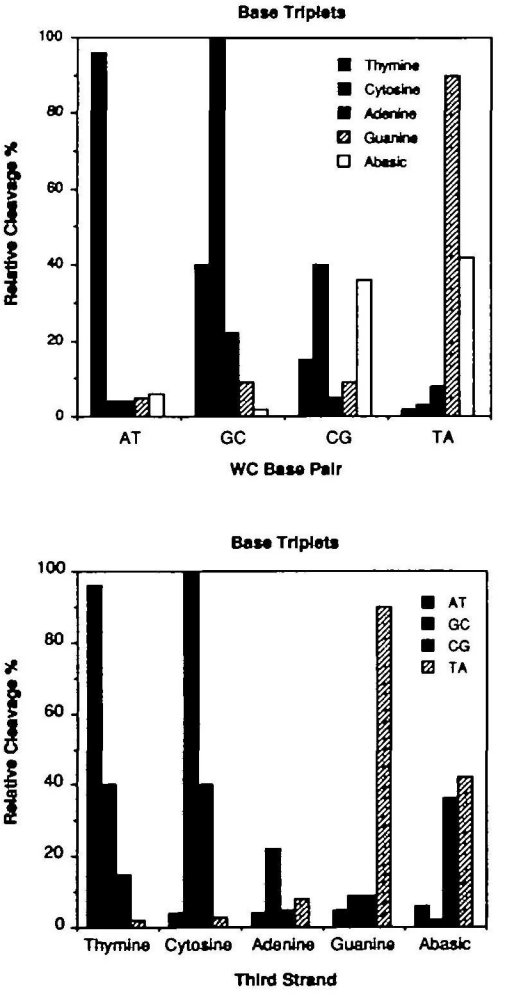

Figure 2. Histograms depicting relative cleavage intensities (normalized) for the twenty base triplets. The data are obtained from scintillation counting and densitometric analysis of three separate autoradiograms (Figure 1). Error for each value is estimated as ±10%.

cleavage resulting from triplexes containing N·TA (N=A,C and T) and N·CG (N=A,G and T) triplet imperfections relative to the complexes with ϕ·TA and ϕ·CG sites suggests that further destabilizing (steric) interactions exist between certain mismatched bases (Fig. 2). In contrast, an abasic site across GC (ϕ·GC) reduces triplex stability to a significantly greater extent than a T·GC triplet. Finally, comparison of the cleavage efficiencies for triplexes containing G·TA and ϕ·TA demonstrates that G contributes a positive interaction across TA base pairs rather than effectively being the most tolerable of triplet imperfections (Fig. 1, lanes 14 and 22 and Fig. 2). This supports our hypothesis that both base stacking and hydrogen bonding are important for stabilizing the G·TA triplet within the pyrimidine motif (5). It should be noted that the stability of the G·TA triplet is dependent on nearest neighbor interactions (5, 15, 16). We would anticipate that the identity of the base triplets near an abasic site will impact the stability of the complex containing such a site.

CONCLUSION

Analogous to duplex DNA, it is apparent that the stability of triple helical complexes is dependent upon base stacking contributions as well as base pairing of its composite bases (8). Removal of a single base in the third strand causes a decrease in overall triplex stability which implies that an abasic site is unlikely to be used as a null position for the recognition of mixed sequence DNA sites by pyrimidine oligonucleotides. More appropriately, an abasic residue in conjunction with other bases and synthetic heterocycles, might serve as a diagnostic control for characterizing the relationship between structure and energetics for the most stable triple helical complexes. Due to limitations in affinity cleaving methodology, additional characterization of the abasic site by direct physical methods such as spectroscopic and calorimetric analyses will be necessary to define the structural basis for these observations.

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